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Checking the depth specificity of a subterranean sampling device in alpine rock debris

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Abstract

Invertebrate communities living in the superficial subterranean environment (MSS, Milieu Souterrain Superficiel) have rarely been studied. One possible reason for this is the labor-intensive sampling method that must be used. The most suitable method to collect these arthropods is by using subterranean sampling devices (SSDs, vertically buried tubes with side holes and a pitfall trap at the bottom). However, there is a possibility that surface-active species that encounter the tube from the outside will follow it into the depths and eventually fall through a hole into the trap at a greater depth, which may influence the recorded species composition within a particular depth range. We examined for the first time the depth specificity of SSDs in the scree layer of a rock glacier in the Swiss National Park. We compared the catches from 1 m long standard SSDs over a period of one year with catches from short sections of standard tubes buried at different depths (15–35, 40–60, 70–90 cm) in the scree layer so that they only captured arthropods at their particular depth range. In total, we found individuals of 11 arthropod species and one gastropod species in the different SSDs. Catches from standard SSDs did not differ in species composition from catches from short tubes buried at a depth range of 40–90 cm, confirming the depth specificity of standard SSDs.

Keywords

MSS, subterranean biology, superficial subterranean habitats, trapping

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Introduction

The superficial subterranean environment (mainly known as «Milieu Souterrain Superficiel», MSS: Juberthie et al. 1980, 1981) poses a particular challenge for the quantitative sampling of arthropods. Sampling in the MSS is usually performed by placing pitfall traps, either buried directly in the ground or installed in perforated metal or PVC tubes (Mammola et al. 2016). Pitfall traps in perforated tubes allow catches at greater depths in the MSS. Pitfall traps in perforated tubes, often referred to as subterranean sampling devices (SSDs; Mammola et al. 2016), are typically 1 m long (exceptionally 2 m; Gers 1992) and buried vertically with sealed tops at the surface (Fig. 1). SSDs are considered the most suitable method for studying arthropods living in superficial subterranean habitats (Mammola et al. 2016). However, this method has been shown to have limitations (Jiménez-Valverde et al. 2015; Jureková et al. 2021). Factors such as the preservant liquid can have a strong influence on catch results (Rendoš et al. 2014; Jureková et al. 2019). In addition, there is the possibility that invertebrates that encounter the PVC tube from the upper soil layer or even from the surface will follow it into the depths and eventually fall through a hole into the pitfall trap at a greater depth (arrows in Fig. 1). This can distort the species composition of the collected samples. The potential bias can be reduced by installing multiple pitfall traps at different depths within the same tube (Rendoš et al. 2012, 2014). Interestingly, the depth specificity of SSDs has not been checked before. It is assumed that the invertebrates captured with SSDs are naturally active in the subterranean habitat at the investigated depth and have not been displaced from the upper layers or from the surface. However, the depth specificity of SSDs is of great importance for the correct interpretation of the sampling results.

In this study, we examined the depth specificity of SSDs in the scree layer of the Valletta rock glacier in the Swiss National Park. Rock glaciers provide cold and stable habitats for terrestrial invertebrates and are considered climate refugia for mountain biodiversity (Brighenti et al. 2021). Although rock glaciers are severely threatened by global warming, arthropod communities in the scree layer have rarely been studied (Gobbi et al. 2014). We buried short sections of standard tubes at different depths in the scree layer of a rock glacier in close proximity to standard SSDs. Using this approach, we aimed to answer the following questions: (1) Do catches of standard SSDs with holes at 40–90 cm represent the invertebrate community present at the corresponding depth of the scree layer? (2) Do standard SSDs attract invertebrates from the surface layer of rock glacier?

Material and methods

Sampling was carried out in the dolomite scree layer of the Valletta rock glacier in the Swiss National Park (SNP) in the Eastern Alps, Grisons, Switzerland (Fig. 2). Rock glaciers are lobe or tongue-shaped landforms composed of a mixture of ice and frozen rock debris that form through gravity-driven creep (Reinosch et al. 2021). The Valletta rock glacier has a north-facing tongue, is 700 m long and 350 m wide, and has an average slope of 22% at elevations between 2490 and 2660 m a.s.l. (Manchado et al. 2024).

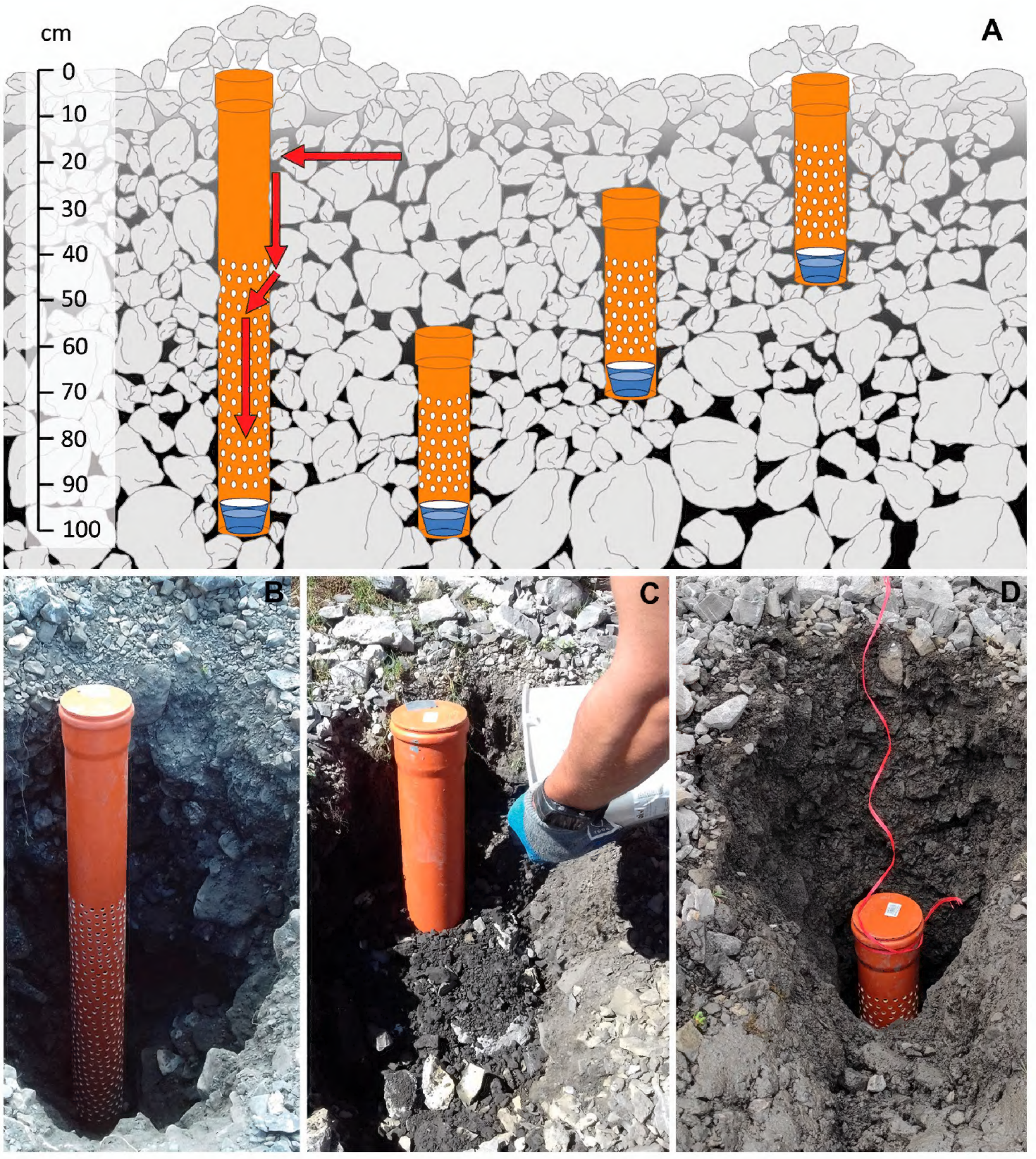

Figure 1. Subterranean Sampling Devices (SSDs) used in the present study **A** schematic representation of the four different SSDs used at each sampling site and the depth at which they are buried. Arrows indicate the possibility that invertebrates may be guided deeper into the ground by the tube **B** photograph of a standard 1-m long SSD placed in the hole before covering **C** process of covering the 1-m standard SSD **D** short SSD placed at a depth of 90 cm, before it was covered with scree material.

In our study, we compared the invertebrates captured in pitfall traps in standard tubes (SSDs) with those captured in pitfall traps in short tubes (hereafter short SSDs) buried at different depths. Standard SSDs consisted of a 1 m long, 11 cm diameter PVC tube buried vertically (Fig. 1). The opening of the tubes was at ground level and closed. At depths between 40 and 90 cm, there were numerous holes with a diameter of 8 mm in the tube through which the invertebrates could enter. We used a plastic

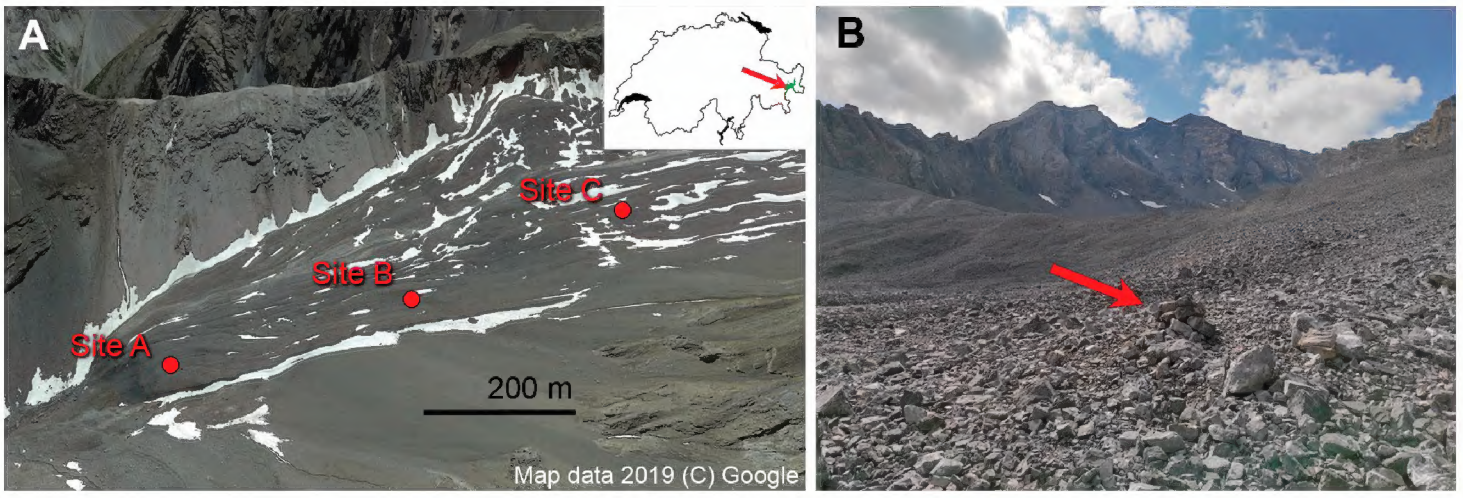

Figure 2. Location of the Valletta rock glacier in the Swiss National Park, eastern Swiss Alps **A** aerial view of Valletta rock glacier **B** small pile of stones covering the standard SSD in site B.

cup with a diameter of 11 cm as a pitfall trap. The pitfall trap containing propylene glycol as a preservative was placed at the bottom of the SSD. Each plastic cup had a threaded rod that allowed it to be raised after the tube was opened. Short SSDs were sections of standard SSDs designed to operate at specific depths (Fig. 1). Short SSDs consisted of a 45 cm long tube with a diameter of 11 cm and, like the standard SSDs, were equipped with a pitfall trap. Short SSDs had holes over a distance of 20 cm (the top 15 cm and bottom 10 cm of the tube had no holes). Buried at different depths, the short SSDs made it possible to collect at a specific depth range.

We buried one standard SSD and one set of three short SSDs, each at three different sites in the scree layer of the Valletta rock glacier (Fig. 2): Site A at 2533 m a.s.l. (46°38'27.20"N, 10°06'9.95"E), site B at 2590 m (46°38'19.01"N, 10°06'3.44"E) and site C at 2645 m (46°38'10.23"N, 10°05'59.25"E). The distance between two sites was 290 m. At each site, we buried the three short SSD at three different depths in the scree layer: one captured invertebrates at a depth range of 15 to 35 cm, the other at a depth of 40 to 60 cm and the third at a depth of 70 to 90 cm. A nylon cord was attached to the short SSDs so that they could be found later (Fig. 1D). Within each site, the standard SSD and the three short SSDs were 3–9 m apart.

Standard SSDs and short SSDs were 341 days in operation (from 16 July 2021 to 22 June 2022). We emptied the traps on the last day, sorted the individuals into taxonomic groups, and preserved them in ethanol. For the present study, individuals from seven taxonomic groups were identified to the species level by experts: Araneae by Ambros Hänggi (juvenile spiders to the family level), Opiliones by Karin Urfer, Diplopoda by José D. Gilgado, Pseudoscorpiones by Jana Christophoryová, Carabidae by Yannick Chittaro and José D. Gilgado, Staphylinidae by Alexander Szallies, and Gastropoda by Bruno Baur and Jörg Rüetschi (Table 1). Voucher specimens were stored in the collection of the Bündner Naturmuseum Chur (BNM), Switzerland.

Statistical analyses were performed in R (R Core Team 2023). To test the depth specificity of the standard SSDs, we compared their catches with those of the short SSDs. If the standard SSDs only catch invertebrates at depths of 40–90 cm, then the species composition should be similar to those of the short SSDs' catches in the

Table 1. Total number of individuals of various invertebrate groups identified at the species or family level that were caught by different SSD types in the scree layer of the Valletta rock glacier over 1 year. Depending on the trap type, the individuals were caught in defined depth ranges (indicated in cm) of the scree layer. For each trap type, data from three sampling sites (A, B and C) were added together.

Class/Order/Family	Taxa	Standard SSDs 40–90	Short SSDs 15–35	Short SSDs 40–60	Short SSDs 70–90
Arachnida/Opiliones/ Phalangiidae	*Dicranopalpus gasteinensis* Doleschall, 1852	1	1	1	0
Arachnida/Araneae/Linyphiiidae	*Mughiphantes variabilis* (Kulczyński, 1887)	1	0	0	0
	Linyphiidae sp. juvenile	1	0	0	0
Arachnida/Araneae/Agelenidae	Agelenidae sp. juvenile	0	0	1	0
Arachnida/Pseudoscorpionida/ Neobisiidae	*Neobisium jugorum* (L. Koch, 1873)	12	2	1	0
Diplopoda/Chordeumatida/ Craspedosomatidae	*Pterygophorosoma alticolum* (Verhoeff, 1894)	195	37	35	31
Diplopoda/Chordeumatida/ Chordeumatidae	*Orthochordeumella pallida* (Rothenbühler, 1899)	1	0	0	0
Insecta/Coleoptera/Carabidae	*Oreonebria bremii* (Germar, 1831)	65	12	17	11
	Oreonebria castanea (Bonelli, 1810)	0	0	0	1
	Leistus montanus rhaeticus Heer, 1837	1	0	0	0
Insecta/Coleoptera/Staphylinidae	*Stenus glacialis* Heer, 1839	1	0	0	0
	Leptusa areraensis Scheerpeltz, 1973	1	0	0	0
Gastropoda/Stylommatophora / Vitrinidae	*Eucobresia glacialis* (Forbes, 1837)	2	0	2	0
Total per trap type		281	52	57	43

40–60 cm and 70–90 cm depth ranges, but different from those of short SSDs in the depth range from 15–35 cm. We used analysis of similarity (ANOSIM) in the "*vegan*" package to examine differences in the invertebrate species composition between the different SSD types (Oksanen et al. 2019). ANOSIM is a nonparametric permutation method that allows comparison of dissimilarities (Bray-Curtis) between-group and within-groups. The procedure calculates *R* statistics ranging from –1 to 1. $R = 0$ indicates completely random grouping, whereas $R = 1$ indicates that all replicates within groups are more similar than all replicates between groups. We also used permutational multivariate analysis of variance (PERMANOVA) to examine our expectations. SSD type was included as factor (three types: standard SSD, short SSD in the 40–60 cm and 70–90 cm depth ranges combined and short SSD at 15–35 cm). The PERMANOVA test was based on 999 permutations of the untransformed raw data using the "*adonis*"function in the *vegan* package (Oksanen et al. 2019). In addition, we applied "*RVAideMemoire*" to perform pairwise comparisons between group levels (depth ranges) with corrections for multiple testing using "*pairwise.MVA.test*".

Results and discussion

In total, we found individuals of 11 arthropod species in the different SSDs, as well as one gastropod species (Table 1). Three arthropod species were found relatively frequently in the traps, whereas all other species were only represented by

1–3 individuals (Table 1). Catches from standard SSDs (depth range 40–90 cm) did not differ in species composition from catches from short SSDs (depth ranges of 40–60 cm and 70–90 cm combined). However, both differed in species composition from the catches of the short SSDs in the depth range of 15–35 cm (ANOSIM: R = 0.34, p = 0.039). PERMANOVA analysis also showed that the species composition varied depending on the depth range at which the SSDs were operated ($F_{2,9}$ = 2.923, p = 0.024). Pairwise comparison of SSDs revealed that only the species composition from short SSDs (depth range 15–30 cm) differed from that of standard SSDs (p = 0.045). In other words, the catches of the standard SSDs represent the invertebrate species that are active at a depth of 40–90 cm in the scree layer. In contrast, a different species composition was found in the short SSDs in the depth range of 15–35 cm. Standard SSDs caught individuals of species not found in short SSDs (depth range of 15–35 cm).

The most frequently caught species was the millipede *Pterygophorosoma alticolum* (68.6% of all individuals), followed by the carabid beetle *Oreonebria bremii* (24.2%) and the pseudoscorpion *Neobisium jugorum* (3.5%). Individuals of the three most abundant species were found in all SSD types, except for *N. jugorum,* which was absent in the short SSDs at 70–90 cm. Notably, seven of the 11 species, the three most abundant and four rarer species, were caught with the short SSDs between 40 and 90 cm (Table 1). Five of these species were also recorded with the standard SSDs. This proves that individuals of these species are naturally active in scree layers at depths of up to 90 cm, either permanently or during seasonal vertical migrations (Nitzu et al. 2014). Thus, standard SSDs do not attract or facilitate the capture of invertebrates from the surface or layer of the scree.

The three most abundant species are high mountain specialists known from rocky environments in the nival zone (Marggi 1992; Szallies and Huber 2014; Kime and Enghoff 2021; Gilgado et al. 2022). The millipede *Pterygophorosoma alticolum* is an alpine-nival species, endemic to the Central Alps, and does not occur below 2000 m a.s.l. (Kime and Enghoff 2021). Its lower elevational limit shifts upward in the study area (Gilgado et al. 2022), reducing its total distribution area and posing a threat to its long-term survival. *Oreonebria bremii* is the alpine carabid beetle with the strongest preference for cold environments (Marggi 1992). This species is endemic to the Central and Eastern Alps. Previously known westernmost populations were recently assigned to a different species (Szallies and Huber 2014). The pseudoscorpion *Neobisium jugorum* is endemic to the Alps with a distribution from Austria to France at elevations of 1700 to 3520 m (Thaler 2003).

In summary, our study showed that standard SSDs do indeed capture individuals of invertebrate species present in the depth range of 40 to 90 cm in the scree layer. Individuals of species that are mainly active on the surface of the scree layer do not seem to follow the tube to enter the SSDs at deeper levels. Our study was carried out at three different sites in the scree layer of a rock glacier. However, the demonstrated depth specificity of the traps may also apply to SSDs in other types of MSS such as scree fields of volcanic or alluvial debris and therefore be of general validity.

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